MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

October–December 2020—Volume 135, pp. 817–823

https://doi.org/10.5248/135.817

Trapelia calyciformis sp. nov. from China

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Abstract—*Trapelia calyciformis* is described from China as a new species. The lichen is characterized by its gray-white thallus, zeorine apothecium with black disc and cracked excipulum, narrow paraphyses branched near the tip, 8-spored asci, aseptate ascospores with one vacuole, and the presence of gyrophoric acid. The specimens examined were deposited in LCUF. A key to the species of *Trapelia* reported in China is presented.

Key words — lichenized fungi, *Trapeliales*, *Trapeliaceae*, taxonomy

Introduction

Trapelia was introduced by Choisy in 1929 and has about 20 species worldwide (Orange 2018). Most taxa are found on rock, rarely on soil and bark (Orange 2018, Aptroot & Schumm 2012, Kantvilas & Elix 2007). The characteristics of this genus are a crustose thallus often with areoles; apotheciate ascomata, slender non-amyloid paraphyses (often branched near the tip but rarely swollen); 8-spored subcylindrical asci with weakly thickened apices; non-septate ascospores that are ellipsoid to subglobose, hyaline, and non-amyloid; and the presence of gyrophoric acid and 5-O-methylhiascic acid (Hertel 1969, 1970; Jaklitsch & al. 2016).

In recent years, much attention has been paid to crustose lichens in China, with many new species and records reported (Aptroot & Seaward 1999; Aptroot & Sipman 2001; Aptroot & Sparrius 2003; Dou & al. 2019; Ren 2015, 2017; Ren & Zheng 2018; Ren & al. 2018; Wang & al. 2015; Yan & al. 2017; Zhou & Ren 2018). Aptroot and his colleagues have reported three species of *Trapelia*—*T. coarctata* (Turner) M. Choisy, *T. involuta* (Taylor) Hertel, and *T. placodioides*

Coppins & P. James. During our study of the genus, two specimens from Jiangxi Province, China differed from the known species and are proposed here as a new species, *T. calyciformis*.

Materials & methods

Specimens. All materials were collected from Jiangxi Province, China, and are deposited in Fungarium of the College of Life Sciences, Liaocheng University, Liaocheng, China (LCUF), and the Lichen Section, Herbarium Mycologium, Chinese Academy of Sciences, Beijing, China (HMAS-L). The macroscopic characters were examined and photographed under an Olympus SZX16 dissecting microscope. The specimens were examined microscopically and photographed under an Olympus BX53 compound microscope. Cortex and medulla were tested using K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), and P (a saturated solution of *p*-phenylenediamine in 95% ethyl alcohol). The lichen substances were detected using standardized thin layer chromatography techniques (TLC) (Orange & al. 2010, Jia & Wei 2016).

DNA extraction, amplification, and sequencing. Molecular protocols followed Dou & al. (2019). The ITS sequence was amplified using the ITS1F and ITS4 primers (Gardes & Bruns 1993, White & al. 1990). Thermocycling conditions were set according to Orange (2018).

The newly generated sequence was submitted to GenBank and aligned with ITS sequences of related taxa selected by a Blast search of closely related ITS sequences and comparison with morphological characters and literature as suggested by Orange (2018) (Fig. 1).

Phylogenetic analysis and sequence comparisons. The ITS sequences of the specimen and 14 selected representatives were aligned by Muscle using MEGA5 (Tamura & al. 2011). The final matrix can be obtained from the corresponding author. A phylogeny was generated using MrBayes on XsEDE (3.2.6) and RAxML-HPC2 on XSEDE (8.2.12) (Miller & al. 2010). The level of confidence in the resulting topological bipartitions was estimated with 1000 bootstrap replicates. All alignment gaps and missing data were excluded during phylogenetic analyses via pairwise sequence comparisons. Absolute distances were estimated using MEGA5, based on p-distance model with all gaps removed from each sequence pair.

Results & discussion

Sequencing & phylogenetic analysis

The partial ITS1 region, complete 5.8S ribosomal RNA gene, and partial ITS2 region of the Jiangxi sample were successfully sequenced; the sequence submitted to GenBank contained 480 base pairs. The sites that were difficult to align were removed from the matrix, and a total of 464 positions were reserved for phylogenetic analyses in the final dataset.

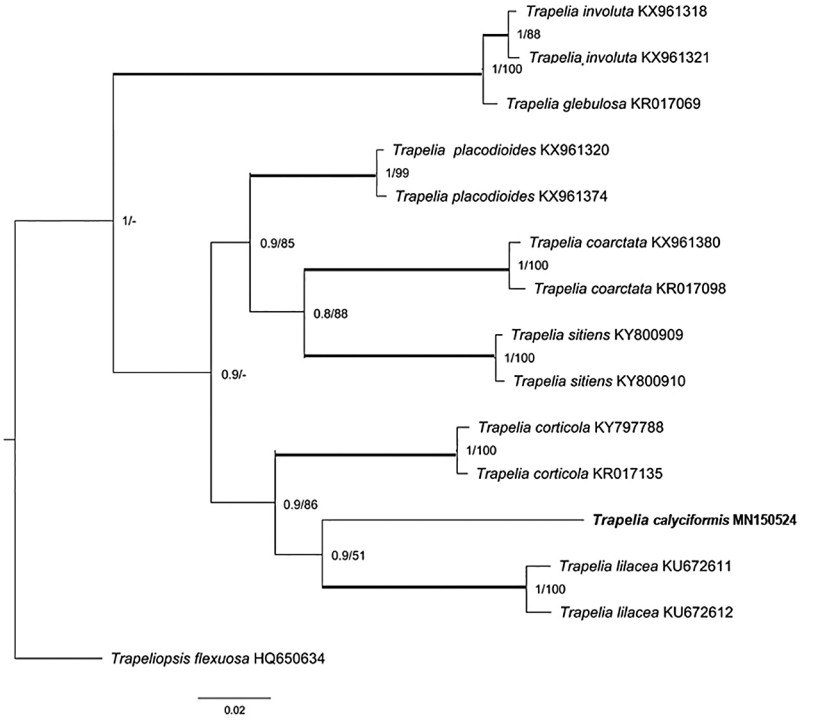

Fig. 1. Phylogenetic relationships inferred from ITS sequences of *Trapelia calyciformis* and closely related *Trapelia* species (with *Trapeliopsis flexuosa* as outgroup). The evolutionary tree was inferred by using the Bayesian method. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Posterior probabilities/ML-bootstrap values >50% are written next to nodes. Branches highly supported by both methods (PP ≥0.95 and ML ≥70%) are indicated with a bold line.

ITS sequence analysis supported a close relationship (98.69% identity) between the new species (*T. calyciformis*) and *T. coarctata* and strongly supported the monophyly of *T. coarctata* (PP = 1; ML = 100%), *T. involuta* (PP = 1; ML = 88%), *T. placodioides* (PP = 1; ML = 99%), *T. sitiens* Orange (PP = 1; ML = 100%), *T. corticola* Coppins & P. James (PP = 1; ML = 100%), and *T. lilacea* Kantvilas & Elix (PP = 1; ML = 100%). *Trapelia. glebulosa* (Sm.) J.R. Laundon clusters together with *T. involuta* (PP = 1; ML = 100%). Our phylogenetic analysis placed the Jiangxi sequences within *Trapelia,* and weakly supported the relationship between *T. calyciformis* and its morphologically closely related species *T. lilacea* (PP = 0.9; ML = 51%) (Fig. 1).

Absolute distances for the aligned sequences of the ITS sequence also support the separation of a new species. In our sequence matrix, distances between infraspecific samples are ≤3 and between species are ≥4, while the distances between *T. calyciformis* and other species are ≥55 (Table 1).

Both molecular and morphological analyses support *T. calyciformis* as a new species.

Table 1. Absolute distances between aligned ITS sequences from *Trapelia calyciformis* and related species. (Gaps ignored in pairwise comparisons.)

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *T. calyciformis* MN150524													
2. *T. placodioides* KX961320	60												
3. *T. placodioides* KX961374	61	**1**											
4. *T. coarctata* KX961380	65	35	36										
5. *T. coarctata* KR017098	66	36	37	**1**									
6. *T. involuta* KX961318	85	68	68	71	72								
7. *T. involuta* KX961321	85	68	68	71	72	**1**							
8. *T. lilacea* KU672611	58	54	54	55	56	80	79						
9. *T. lilacea* KU672612	55	55	56	54	55	82	81	**3**					
10. *T. glebulosa* KR017069	84	65	65	69	70	4	5	78	80				
11. *T. corticola* KY797788	56	48	49	50	51	72	72	54	53	71			
12. *T. corticola* KR017135	56	48	49	50	51	72	72	54	53	71	**0**		
13. *T. sitiens* KY800909	60	39	40	40	41	67	67	58	57	64	52	52	
14. *T. sitiens* KY800910	60	39	40	40	41	67	67	58	57	64	52	52	**0**

* Infraspecific distances are indicated with bold font.

Taxonomy

Trapelia calyciformis Z.F. Jia, **sp. nov.** Fig. 2

FN 570662

Differs from *Trapelia coarctata* by its cracked excipulum.

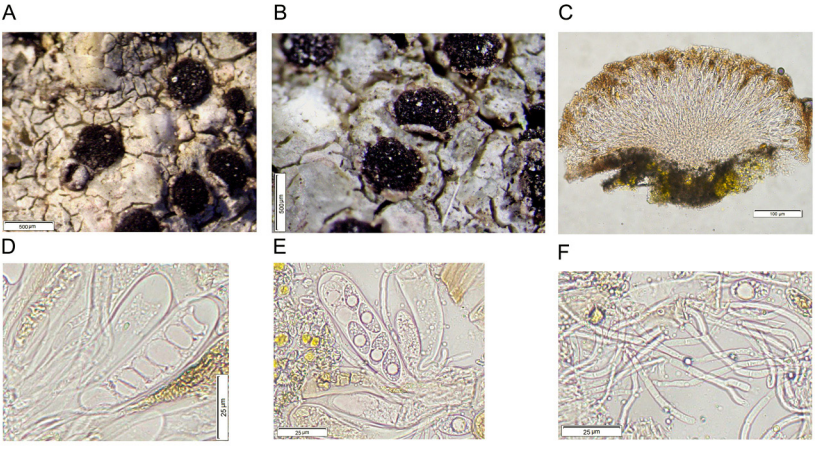

Fig. 2. *Trapelia calyciformis* (holotype, LCUF – Yao JX19016). A. Thallus with apothecia; B. Apothecia with cracked excipulum; C. Cross section of apothecium; D. Asci with thickened apex; E. Ascospores; F. Paraphyses septate and branched near the tip.

Type: China. Jiangxi: Shangrao City, Wuyuan County, Xitou Town, Jiangling Scenic Area, 29°27′N 118°01′E, alt. 392 m, on rock, 16/III/2019, Z.T. Yao JX19016 (**Holotype**, LCUF; **Isotype**, HMAS-L 0144251; GenBank MN150524).

Etymology: From the Latin *calyciformis* meaning "calyx-shaped," referring to the shape of the cracked excipulum.

Thallus crustose, saxicolous, off-white, dull. Algae chlorococcoid. Apothecia zeorine, orbicular, sessile, 0.4–0.5 mm diam.; disc convex, black, epruinose; exciple brown; epithecium brown, 21–29 µm tall; hymenium hyaline, without oil droplets, I–, paraphyses branched near the tip, 1.5–2.5 µm diam.; hypothecium light brown. Asci clavate, 80–90 × 15–17 µm, 8-spored. Ascospores 15.2–22(–28) × 7.2–10.7 µm, non-septate, ellipsoid, hyaline. Pycnidia not observed.

Chemistry: Thallus UV–, C+ red, K–, P–. Gyrophoric acid detected with TLC.

Ecology & distribution: On rocks. Known only from the type locality.

Additional specimen examined: **CHINA**. **Jiangxi**: **Shangrao City**, Wuyuan County, Xitou Town, Jiangling Scenic Area, alt. 392 m, on rock, 16/III/2019, Z.T. Yao JX19017 (LCUF).

Remarks: *Trapelia calyciformis* morphologically resembles *T. coarctata*, which differs by its white mound-like apothecia in its early stage and intact excipulum without cracks when mature (Orange 2018, Brodo & Lendemer

2015); however, *T. calyciformis* is very well separated from *T. coarctata* in ITS sequences. *Trapelia calyciformis* is also similar morphologically to *T. glebulosa,* which is distinguished by ITS sequence differences, its biatorine apothecia, and a thallus that does not appear as a single, coherent unit but instead comprises distinct areoles with abruptly thickened margins (Orange 2018; Brodo & Lendemer 2015). Despite the close relationship between *T. calyciformis* and *T. lilacea* in the ITS tree, the basal node is only weakly supported. Moreover, *T. calyciformis* differs distinctly from *T. lilacea* in morphology and chemistry: *T. lilacea* is distinguished by its wider ascospores (9–15 µm diam.), sunken concave thallus disc, and 5-O-methylhiascic acid as its primary lichen substance (Kantvilas & Elix 2007).

Key to the species of *Trapelia* known from China

1. Soralia present ..... *T. placodioides*
1. Soralia absent 2
2. Mature apothecia without thalline exciple, 5-O-methylhiascic acid (major) ..... *T. involuta*
2. Mature apothecia with thalline exciple, gyrophoric acid (major) 3
3. Thalline exciple margin cracking into multiple triangular lobes ..... *T. calyciformis*
3. Thalline exciple margin not cracking into multiple triangular lobes .... *T. coarctata*

Acknowledgments

This study was supported by the National Natural Science Foundation of China (31700018 & 31750001) and Doctoral Research Foundation of Liaocheng University (318051813). The authors are grateful to the presubmission reviewers Prof. Qiang Ren (State Key Laboratory of Mycology, Institute of Microbiology, CAS) and Dr. Lei Lü (College of Food Science and Engineering, Qilu University of Technology, China) for reading and improving the manuscript.

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